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Primary Small Cell Carcinoma of the Esophagus: Flow Cytometric Analysis and Immunohistochemical Staining for the p53 Protein and Proliferating Cell Nuclear Antigen

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Background and Objectives: The purpose of this study was to determine the clinical course and effects of histopathologic characteristics of specific tumors including DNA contents and immunohistochemical aspects in patients with small cell carcinoma of the esophagus.
Methods: Medical records of 4 patients who presented with small cell carcinoma of the esophagus were retrospectively reviewed.
Results: DNA aneuploidy was observed in 2 cases. Staining for the p53 product was positive in all cases. The average proliferating cell nuclear antigen (PCNA) labeling rate (LR) was 77.6% (64.0–90.8%). The estimated median survival was 42 days for all patients. Distant metastases were observed in 2 of the 4 patients.
Conclusions: Higher PCNA LR of small cell carcinoma may be an unfavorable characteristic of biological behavior. Patients with disseminated disease should have symptomatically palliative operation combined with chemotherapy. *J. Surg. Oncol. 1998;68:246–249.*

KEY WORDS: **small cell carcinoma; esophagus; p53; PCNA; flow cytometry**

INTRODUCTION

Small cell carcinoma may occur in any portion of the alimentary tract, but, in fact, it occurs quite infrequently. [1]. Like small cell carcinoma of the lung, small cell carcinoma of the alimentary tract has an aggressive feature, and patient survival rate is poor [1,2]. Thus, it seems important to analyze characteristics of small cell carcinoma as related to patient prognosis.

In the current study, we histologically analyzed lesions of small cell carcinoma of the esophagus including immunohistochemistry and flow cytometry. It is widely known that proliferative activity, as measured by immunohistochemistry using antibody against proliferation-associated antigens, is an important prognostic feature of malignant tumors [3–5]. Proliferating cell nuclear antigen (PCNA) is an auxiliary protein of DNA polymerase-δ, and the level of synthesis correlates directly with rates of cellular proliferation and DNA synthesis [6]. The p53 gene is also believed to play an important role in the control of cell proliferation and tumor progession [7].

PATIENTS AND METHODS

The medical and pathologic records of all of the patients with primary small cell carcinoma of the esophagus who were treated at the Department of Surgery, Toyama Prefectural Central Hospital, Toyama, Japan, from 1981 to 1997 were reviewed. A series of 4 patients is included and follow-up is available from all patients.

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TABLE I. Summary of Patients Who Underwent Operation for Small Cell Carcinoma of the Esophagus

Patient no.	Age (years)	Site	Size (cm)	DNA	PCNA (%)	p53	TNM classification and operation	Survival (days)	Recurrence	Adjuvant therapy
1	49	Middle	9.6	Diploid	90.8	+	T2N1M1: stage IV Total esophagectomy	103 (dead)	Liver, lung	Chemotherapy
2	65	Lower	6.0	Diploid	64.0	+	T3N1M1: stage IV Total esophagectomy	22 (dead)	Pleura	None
3	62	Middle	2.5	Aneuploid	75.2	++	T1N0M0: stage I Total esophagectomy	281 (dead)	Lung, bone	None
4	66	Middle	5.0	Aneuploid	80.4	++	T4N1M0: stage III Total esophagectomy	42 (dead)	Pleura	None

They were all males, aged 49–66 years (mean: 60.5 years).

Microscopic sections of the entire resected carcinoma were prepared from step-sectioned blocks 5 mm in width, and after staining with hematoxylin-eosin (HE), histologic features such as the depth and extent of the main or accessory lesions, involvement of either the lymphatic or vascular vessels, and lymph node metastasis were evaluated. Adequate clinical data and follow-up information were obtained for all 4 cases with small cell carcinoma. The TNM classification of malignant tumors of the International Union Against Cancer was used and histologically confirmed from resected specimens.

Immunohistochemical Methods for Staining p53 and PCNA

Histology of all cases was reviewed and representative sections from 2–5 different areas of each tumor were selcted for immunohistochemistry. These were deparaffinized in xylene, transferred to 95% ethanol, and air-dried. The monoclonal rabbit anti-p53 antibody D0-7 raised against the full-length human p53 protein was used as a primary antibody. Briefly, the slides were incubated overnight at 4°C with monoclonal anti-p53 antibody and diluted 1:750. The sections were washed and treated consecutively for 30 min with biotinylated swine anti-rabbit antibody (Dakopatts, Glostrup, Denmark) diluted 1:400 in phosphate-buffered saline (PBS) and streptavidin horseradish peroxidase (HRP)-conjugated reagent (Dako Ltd., UK) diluted 1:800, at room temperature. Peroxidase was visualized using a 0.05% solution of diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) and 0.1% solution of nickel chloride in PBS containing 0.03% hydrogen peroxide (10 min). Finally, the slides were lightly stained in hemalum (Mayer's, Merck, Poole, UK). Replacement of the primary antibody with normal rabbit serum was used as a negative control. Specific nuclear staining of cells (regardless of proportion) was considered positive for abnormal.

Anti-PCNA monoclonal antibody PC10, a mouse IgG2a isotype, was obtained from Dakopatts (Copenhagen, Denmark). Immunohistochemical staining with PCNA antibody was performed in the same manner as described for p53 antibody. All nuclei that showed intranuclear reactivity against PC10 were regarded as positive. From 1,000 to 2,000 cells were counted to determine the PCNA labeling rates (LRs).

Flow Cytometry (FCM)

Each paraffin-embedded tissue block was then sliced into five 50 μm sections, from which cells were isolated by the method of Schutte et al. [8]. The specimen was first dewaxed with xylene at 37°C for 1 hr with the solvent replaced twice, and progressively rehydrated with 100%, 95%, 70%, and 50% solutions of ethanol at room temperature for 30 min. The tissue was then washed with distilled water for 20 min and incubated in a 0.25% solution of trisodium citrate buffer (3 mM trisodium citrate, 1.5 mM spermine, 0.5 mM TRIS, and 0.2% Triton X, pH 7.6) at 37°C, filtered through a 40 μm mesh, and centrifuged at 1,000 rpm for 10 min. The supernatant was discarded. The sediment was washed twice with PBS. It was finally treated with 0.1% RNase (Sigma) and a fluorescent dye containing 50 μg/ml of propidium iodide (PI). The number of cells was adjusted to 100,000 cells/ml.

FCM analysis was performed with FACScan flow cytometer (San Jose, CA). The DNA ploidy of a cell population was deemed diploid when the cells exhibited a single G_0G_1 peak and a DNA index of 1.0. Cell populations showing 2 discrete G_0G_1 peaks were deemed aneuploid. Human blood lymphocytes were used as the internal standard to identify the diploid population. The DNA index was calculated by comparing the channel ratio of G_0G_1 populations. A histogram was prepared using a minimum of 10,000 cells.

RESULTS

Clinical Presentation

In this study, the numbers of patients by TNM stage were as follows: 1 in stage I, 1 in stage III, and 2 in stage IV. One of the patients underwent curative resection and 3 others underwent non-curative resection because of metastases ($n = 2$) or local invasion ($n = 1$) (Table I).

Pathologic Characteristics

Histologic sections were reviewed to confirm the diagnosis of small cell carcinoma. Immunohistochemical testing was done when lymphoma or carcinoid tumor had been included in the differential diagnosis. The tumor size ranged from 2.5 to 9.6 cm (mean: 5.8 cm) in the maximum diameter. The average PCNA LR was 77.6% (range: 64.0–90.8%). DNA diploidy was observed in 2 cases and DNA aneuploidy in 2 cases. Staining for the p53 product was positive in all (Table I).

Tumor Mortality and Recurrence

The estimated median survival was 42 days for all patients. Primary or secondary, all patients presented with distant or lymph node metastases. Table I shows the sites of recurrence. Distant metastases were observed in all of the patients, with the lung involved in 2, the pleura in 2, the liver in 1, and the bones in 1. Metastases also involved the lymph node in 3 patients. One patient (No. 3) underwent curative resection. He was, however, dead of disease at 281 days with recurrence after the initial surgery.

DISCUSSION

The alimentary tract is a major site of carcinoma in humans. There are, however, great differences in incidence among the component sites from the esophagus to the anus. Furthermore, a number of the histologic types of tumors at these sites differ in their incidence and prognosis. Small cell carcinoma of the alimentary tract with oat cell-like histology has been observed previously [9–11]. Like small cell carcinoma of the lung, small cell carcinoma of the alimentary tract has an aggressive feature, and the survival rate of patients is poor [1,12].

In esophageal tumors, squamous cell carcinoma is the most common type of esophageal malignancy. Small cell carcinomas account for 0.7% of the malignant tumors [2]. Thomas and Sobin [2] reported that no patients with small cell carcinoma of the esophagus survived at 5 years. Small cell carcinoma of the esophagus is associated with rapid growth and patients usually present with widespread metastatic disease. Survival is on the order of 1–10 months in our series. One patient (No. 3) in our series who had undergone curative resection demonstrated widespread metastases postoperatively. Craig et al. [13] reported 7 patients who underwent operation, either alone or with adjuvant chemotherapy or radiotherapy, with a mean survival of 20 months (range: 2 weeks to 96 months). According to their report, 9 patients had disseminated disease when they were first seen and treated by palliative chemotherapy, radiotherapy, or no therapy, with a mean survival of 4.8 months (range: 2–9 months). Huncharek and Muscat [14] reported 13 patients who received no operation and underwent radiotherapy, either alone or with chemotherapy, with overall survival of only 7 months (range: 3–24 months).

Most of the reported cases, including our series, showed metastasis in the lymph nodes or in distant organs at the time of diagnosis, because of their highly aggressive behavior. In this study, we have assessed the DNA ploidy and the proliferative activity by immunohistochemical analysis, such as p53 expression and PCNA LR, to specify the differences in biologic behavior of small cell carcinoma.

The FCM study of nuclear DNA patterns is available at present. It provides useful prognostic information on a wide range of human neoplasms [15–17]. DNA aneuploidy has been found to be correlated with growth pattern, and some investigators have shown that it is correlated with a poor prognosis in alimentary carcinoma cases [15,18,19]. In the current study, DNA aneuploidy was observed in 2 cases. It has been postulated that p53 might act as a tumor suppressor gene, because its mutation or allelic deletion plays an important role in the development of many human cancers. Normal p53 induces apoptosis in detecting the damage of DNA, while mutant p53 and allelic deletion of p53 suppress it. Immunohistochemical detection of p53 has been considered to be associated with p53 mutation in situ [20]. On the other hand, it is known that proliferative activity, as measured by immunohistochemistry using PCNA, is an important prognostic feature of malignant tumors [21–23]. The current report also analyzed the PCNA LR of small cell carcinoma. The average PCNA LR was 77.6%, which was demonstrated to be higher than in other types of carcinoma [21–23], which supports the evidence for the aggressiveness of small cell carcinoma. In this study, staining for the p53 product was positive in all cases.

Consequently, our study suggests that the higher PCNA LR of small cell carcinoma may be an unfavorable characteristic of biological behavior. Additionally, we think that patients with disseminated disease should be symptomatically palliative operation combined with chemotherapy, because the non-curative resection implied a less favorable prognosis.

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